

EXPRESS MAIL CERTIFICATE

EU 039138725US

Date 2/20/02 Label No. \_\_\_\_\_

I hereby certify that, on the date indicated above I deposited this paper or fee with the U.S. Postal Service and that it was addressed for delivery to the Commissioner of Patents and Trademarks, Washington, D.C. 20231 by "Express Mail Post Office to Addressee" service.

A. Diulio A. Diulio  
Name (Print) Signature

5408/1I069US1

**STABLE PRESERVATIVE FORMULATIONS COMPRISING  
HALOPROPYNYL COMPOUNDS AND BUTOXIDIGLYCOL SOLVENT**

This application claims priority under 35 U.S.C. §119(e) of provisional application serial no. 60/271,760, filed February 26, 2001, which is hereby incorporated by reference.

**FIELD OF THE INVENTION**

10 This invention relates to stable, liquid preservative formulations comprising (a) a halopropynyl compound and (b) a butoxydiglycol solvent, and optionally (c) (i) an alkanol substituted dialkylhdantoin formaldehyde donor, (ii) an isothiazolone derivative, and (iii) a stabilizer. The invention is also directed to methods of using the preservative formulations for

inhibiting or retarding the growth of microbes, including bacteria and/or fungi.

## BACKGROUND OF THE INVENTION

The need for effective and economical preservative compositions is well known. Many products, including personal care products such as shampoos, creams, lotions, cosmetics, and soaps, household products such as laundry detergents, hard surface cleaners, fabric softeners, and various industrial products, require preservatives to protect against contamination and growth of bacteria or fungi. In particular, personal care product compositions are a nutrient-rich media which benefit from the incorporation of preservatives to control the growth of microorganisms and to prevent spoilage. Generally, the shelf life of these products depends on the resistance to microbial spoilage of components contained therein. It is therefore desirable to formulate a preservative which controls microbial contamination in personal care products, household products, and industrial products.

For the foregoing applications, the demand for stable, broad-spectrum preservatives has increased. For example, formaldehyde and isothiazolone derivatives have been shown to be highly effective biocidal preservatives. U.S. Patent 3,987,184, to Foelsch discloses 1,3-dimethylol-5,5-dimethylhydantoin (DMDMH) as a useful formaldehyde donor compound for the preservation of personal care products, cosmetics, and household and industrial products. Combinations of formaldehyde donors and halopropynyl compounds (e.g., 3-iodo-2-propynylbutyl carbamate (IPBC)) have achieved considerable commercial success. Such synergistic combinations have been described in, e.g., U.S. Patent No. 4,844,891.

However, governmental regulations currently demand products containing a relatively low amount of free formaldehyde. In the case of DMDMH, improved formulations

and processing has resulted in compositions which contain very low amounts of free formaldehyde. (See U.S. Patent 5,405,862.)

Iothiazolone is highly toxic and very unstable under most circumstances, such as when present in water or other reactive molecules. To make the compound stable, large amounts of cationic salts are added and the isothiazolone is diluted (usually to about 14% or less). While under these conditions, isothiazolone is stable at room temperature at low pH (from 1-4). During storage and manufacturing conditions the temperature and pH may increase, causing isothiazolone to become unstable.

While highly useful for controlling bacteria, fungi and other contaminating microbes in end-use products, the instability of isothiazolone under less than ideal conditions results in a marked loss of activity. Thus, it would be advantageous to provide a preservative system that contains isothiazolone which is stable at a broad range of temperature and pH.

Additionally, under very acidic pH conditions, some end-use products such as personal care products cannot be easily formulated with isothiazolone. At less acidic pH levels, comparatively greater amounts of isothiazolone are needed in a preservative formulation because there is some loss of activity. Preferably, a preservative system should be easy to formulate and have low levels of stable isothiazolone, so that it is nontoxic and non-irritating, but still provides biocidal activity. Stable isothiazolone formulations which are effective at less acidic pH levels have not heretofore been easily obtained.

Furthermore, effective broad spectrum preservative systems that contain formaldehyde donors with low free-formaldehyde and stable isothiazolone for use in applications at less acidic pH levels, free of cationic salts and which are non-toxic, are not readily available.

U.S. Patent No. 6,121,302 describes highly stable, preservative formulations having broad spectrum biocidal activity, which can be prepared by admixing one or more isothiazolones with a formaldehyde donor, such as alkanol-substituted dimethylhydantoins, and a hydantoin as a stabilizer for isothiazolone.

5 In addition, stable liquid preservative formulations having broad spectrum antimicrobial properties can be prepared by mixing alkanol-substituted dimethylhydantoins and antimicrobial halopropynyl compounds. For example, U.S. Patent No. 6,143,204 describes preservative formulations comprising an alkanol-substituted dimethylhydantoin, an iodopropynyl compound, a stabilizer comprising hydantoin or urea or derivatives thereof, and a hydroxyl solvent.

## SUMMARY OF THE INVENTION

Applicants have discovered that the combination of a halopropynyl compound and butoxydiglycol solvent results in an unexpected synergistic antimicrobial formulation demonstrating synergistic properties, i.e. the formulation demonstrates antimicrobial properties which are greater than the additive fungicidal properties of halopropynyl and butoxydiglycol used alone.

In one embodiment, the invention is directed to stable, broad spectrum liquid preservative formulations comprising a halopropynyl compound and butoxydiglycol solvent. In preferred embodiments, the halopropynyl compound is an iodopropynyl.

20 In additional embodiments, the invention is directed to stable, broad spectrum liquid preservative formulations comprising, (a) a halopropynyl compound, (b) an alkanol

substituted dialkylhydantoin formaldehyde donor, and (c) butoxydiglycol. In preferred embodiments, the alkanol substituted dialkylhydantoin is dimethylol dimethylhydantoin, and the halopropynyl compound is an iodopropynyl compound, for example a halopropynyl carbamate such as iodo-2-propynylbutyl carbamate.

5 In another embodiment, the invention is directed to stable, broad spectrum liquid preservative formulations comprising (a) a halopropynyl compound, (b) an isothiazolone derivative, (c) an alkanol substituted dialkylhydantoin formaldehyde donor, (d) a hydroxyl solvent, and optionally (e) a stabilizer. Preferred halopropynyl compounds are iodopropynyl; preferred isothiazolone derivatives are 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; a preferred alkanol substituted dialkylhydantoin is dimethylol dimethylhydantoin; and a preferred solvent is butoxydiglycol.

10 In additional embodiments, the invention is directed to a personal care product, household product, or industrial product comprising an antimicrobial effective amount of one of the preservative formulations of the invention. A preferred personal care product is a protein shampoo. Preferred industrial products are pulp and paper products.

15 The invention is also directed to methods for killing or retarding the growth of microbes (such as bacteria and/or fungi) in a composition susceptible to growth, comprising adding to said composition an effective amount of a preservative formulation of the invention.

20 The invention is also directed to methods of making liquid preservative formulations, comprising dissolving a halopropynyl compound in butoxydiglycol solvent. Additional water-based additives can be added to the halopropynyl-butoxydiglycol solution.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

As used herein, the term “biocidal” means capable of killing microorganisms, including bacteria, yeast, algae and fungi.

5 As used herein, the term “antimicrobial” means capable of killing and/or inhibiting growth of microorganisms, including bacteria, yeast, algae and fungi.

As used herein, the terms “antifungal” or “fungicidal” are used interchangeably, and mean capable of killing and/or inhibiting the growth of fungi.

10 As used herein, the terms “antibacterial” or “bactericidal” are used interchangeably, and mean capable of killing and/or inhibiting the growth of bacteria.

As used herein, the term “hydroxyl solvent” means a solvent containing one or more hydroxyl groups, and includes mono-, di- and polyhydroxy alcohols. Preferred monohydroxy alcohols are C<sub>1-7</sub> alcohols; preferred dihydroxy alcohols are C<sub>1-8</sub> diols; preferred polyhydroxy alcohols are C<sub>2-8</sub> polyols.

15 As used herein, the term “alkyl” refers to a straight or branched hydrocarbon group having a single radical.

As used herein, the term “alkenyl” refers to a straight or branched hydrocarbon group that contains from a single radical, and has one or more double bonds between carbon atoms.

20 As used herein, the term “cycloalkyl” means a non-aromatic mono or multicyclic hydrocarbon ring system of from 3 to 12 carbon atoms having a single radical.

As used herein, the term "cycloalkenyl" means a non-aromatic mono or multicyclic hydrocarbon ring system of from 3 to 12 carbon atoms having a single radical and at least one carbon to carbon double bond.

5 As used herein, the term "alkoxy" refers to the group -O-alkyl, wherein the alkyl moiety is as defined above.

As used herein, the term "aryl" means an aromatic carbocyclic ring structure having from about five to about fifteen carbon atoms. An aryl group may be a fused or polycyclic ring system.

10 As used herein, the term "heterocyclyl" means a closed ring structure having from about five to about fifteen atoms in the ring, in which one or more of the atoms in the ring is an atom other than carbon, such as oxygen, nitrogen or sulfur. A heterocyclic group may be aromatic or non-aromatic, and may be a fused or polycyclic ring system.

### Halopropynyl Compounds

15 A first active component of the stabilized preservative system of the invention is a halopropynyl compound, a fungicidally active iodopropynyl derivative. Suitable halopropynyl compounds are within the general structure (I):

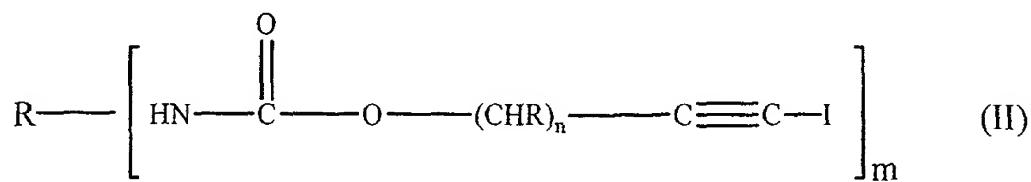


wherein Y is a halogen, preferably iodine and X can be (1) oxygen which is part of an organic functional group; (2) nitrogen which is part of an organic functional group; (3) sulfur which is part of an organic functional group; or (4) carbon which is part of an organic functional group.

The functional group of which oxygen is a part is preferably an ether, an ester, or a carbamate group. The functional group of which nitrogen is a part is preferably an amine, an amide, or a carbamate group. The functional group of which sulfur is a part is preferably a thiol, a thione, a sulfone, or a sulfoxide group. The functional group of which carbon is a part is preferably an ester, a carbamate or an alkyl group.

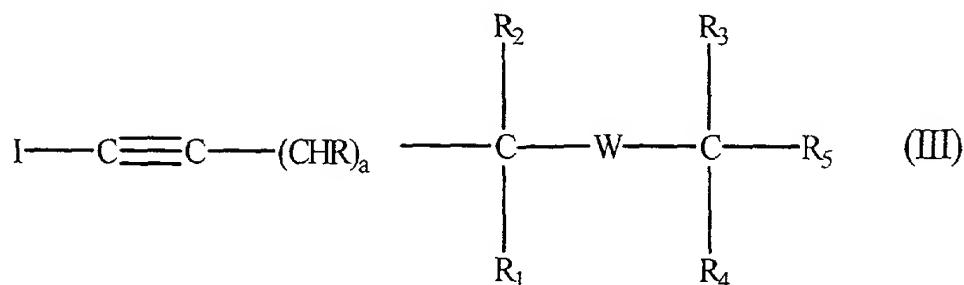
Examples of compounds which may be used as the halopropynyl compound of this invention are especially the fungicidally active iodopropynyl derivatives. In this regard, please see U.S. Pat. Nos. 3,923,870, 4,259,350, 4,592,773, 4,616,004, 4,719,227, and 4,945,109, the disclosures of which are herein incorporated by reference. These iodopropynyl derivatives include compounds derived from propynyl or iodopropynyl alcohols such as esters, ethers, acetals, carbamates and carbonates and the iodopropynyl derivatives of pyrimidines, thiazolinones, tetrazoles, triazinones, sulfamides, benzothiazoles, ammonium salts, carboxamides, hydroxamates, and ureas. Preferred among these compounds is the halopropynyl carbamate, 3-iodo-2-propynyl butyl carbamate (IPBC).

Preferred halopropynyl compounds are included within the broadly useful class of compounds having the generic formulas such as II and III shown below:



wherein:

R is selected from the group consisting of substituted and unsubstituted alkyl, aryl, and alkylaryl groups having from 1 to 20 carbon atoms; and  
m and n are independent integers from 1 to 3; and



wherein:

R<sub>1</sub> and R<sub>2</sub> are defined as R<sub>3</sub> and R<sub>4</sub> below or are joined to form a cycloalkyl, cycloalkenyl, aromatic or a heterocyclic ring containing an oxygen, nitrogen or sulfur atom or an alkoxy, amino, carboxyl, halo, hydroxyl, keto or a thiocarboxyl-substituted derivative thereof;

R<sub>3</sub> and R<sub>4</sub> are independently selected from (A) hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, aryl, a heterocyclic ring containing an oxygen, nitrogen or sulfur atom, alkoxy, amino, carboxyl, halo, hydroxyl, keto or a thiocarboxyl and (B) substituted derivatives of the alkyl, cycloalkyl, alkenyl, cycloalkenyl, aryl and the heterocyclic ring wherein the substitutions are alkyl, cycloalkyl, alkenyl, cycloalkenyl, aryl, alkoxy, amino, carboxyl, halo, hydroxyl, keto or a thiocarboxyl;

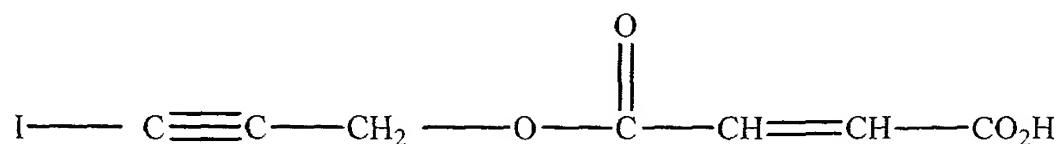
a is 0 to 16;

15 W may be a single bond, oxygen, NR<sub>5</sub>, or (CR<sub>6</sub>R<sub>7</sub>)<sub>m</sub>, wherein R<sub>5</sub> is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, aryl or a heterocyclic ring containing an oxygen, nitrogen or sulfur atom or a substituted derivative of alkyl, cycloalkyl, alkenyl, cycloalkenyl or aryl groups

wherein the substitutions are alkyl, cycloalkyl, alkenyl, cycloalkenyl, aryl, alkoxy, amino, carboxyl, halo, hydroxyl, keto, or a thiocarboxyl wherein R<sub>6</sub> and R<sub>7</sub> are defined as R<sub>3</sub> and R<sub>4</sub> above and m is an integer from 1 to 12. The above definition of R<sub>5</sub> includes, among other things, an aminoalkyl group.

5 The heterocyclic rings referred to in the above definitions may contain from 5 to 8 members, the alkyl or cycloalkyl groups from 1 to 18 atoms, the alkenyl or cycloalkenyl groups from 2 to 18 carbon atoms, and the aryl groups from 6 to 10 members.

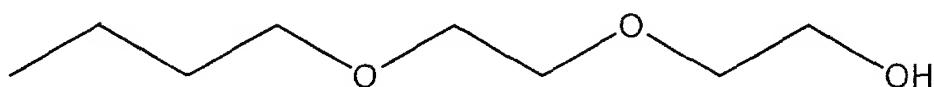
In formula III, when R<sub>1</sub> and R<sub>2</sub> are hydrogen, R<sub>3</sub> and R<sub>4</sub> are carbonyl, R<sub>5</sub> is - CH=CH-CO<sub>2</sub>H; a is equal to 0; and W is oxygen, the compound is iodopropynyl maleate,



Other compounds include the mono-iodopropynyl esters of anhydrides such as succinic and phthalic as well as the following anhydrides: ethylenediamine tetraacetic dianhydride, 3,3-dimethylglutaric anhydride, S-acetylmercaptosuccinic anhydride, dichloromaleic anhydride, 2-dodecen-1-yl succinic anhydride and cis-5-norbornene-endo-2,3-dicarboxylic anhydride. Where hydrophilicity is desired, the sodium salts may be used because of their extremely high water solubility. Preferred carboxylic acid anhydrides include succinic, itaconic, phthalic, tetrachlorophthalic, and diglycolic anhydride. Such compounds are defined in U.S. Patent 4,844,891 and 5,073,570.

**Solvents**

The preferred solvent used in formulations of the invention is butoxydiglycol, also known as 2-(2-butoxyethoxy)ethanol or diethylene glycol-n-butyl ether. Butoxydiglycol is the nomenclature used by the Cosmetic, Toiletry and Fragrance Association. Butoxydiglycol has the structure:



The inventors have now found that butoxydiglycol demonstrates synergistic antimicrobial properties when used in combination with halopropynyl compounds. Thus, the formulations containing a halopropynyl and butoxydiglycol demonstrate antimicrobial properties which are greater than would be expected from the addition of the known antifungal properties of halopropynyl and butoxydiglycol alone.

Formulations containing fungicidal halopropynyl compounds and butoxydiglycol also unexpectedly demonstrate a broad spectrum antimicrobial property, including bactericidal and fungicidal activity.

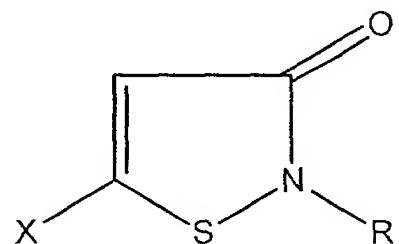
Some additional solvents which may also be present include water and other hydroxyl solvents. Hydroxyl solvents include mono-, di- and polyhydroxyl alcohols. For example, monohydroxyl alcohols having from about 1 to 7 carbon atoms, most preferably ethanol and propanol, may be used. Dihydroxyl alcohols (e.g., glycols) such as C<sub>2</sub> to C<sub>8</sub> diols, may also be used. Aside from butoxydiglycol, other glycols, such as C<sub>2</sub> to C<sub>8</sub> diols are advantageous. Other compounds which can be used include dipropylene glycol, glycerin,

diglycerin, PPG-9, PPG-2-buteth-2, butoxypropanol, PPG-2 butyl ether, glycereth-7, isopentyldiol, myristyl myristate, phenoxy ethanol, and benzyl alcohol.

In the liquid preservative formulation embodiment of the invention comprising (a) a halopropynyl compound, (b) an isothiazolone derivative, and (c) an alkanol substituted dialkylhydantoin, the solvent may be any hydroxyl solvent. A preferred hydroxyl solvent is a butoxydiglycol solvent.

### Isothiazolones

A second antifungal or antibacterial active component of the preservative formulation is one or more antimicrobial isothiazolone derivatives, such as 3-isothiazolones having formula IV:



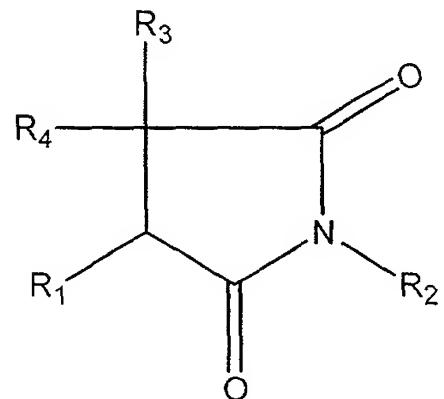
(IV)

wherein X is hydrogen or halogen, preferably chlorine, and R is an alkyl chain of from 1 to 22 carbon atoms. Preferred isothiazolone derivatives include 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) and 2-methyl-4-isothiazolin-3-one (MI), and mixtures thereof (e.g., CMI/MI). Other 3-isothiazolones can be used in the invention, including 4-chloro-2-methyl-4-isothiazolin-3-one, dichloroisothiazolones such as 4,5-dichloro-2-methyl-4-isothiazolin-3-one, bromoisothiazolones

such as 5-bromo-2-methyl-4-isothiazolin-3-one, n-octylisothiazolones such as 2-n-octyl-4-isothiazolin-3-one, and benzisothiazolone.

### Formaldehyde Donors

A third active component of the stabilized preservative formulation is a formaldehyde donor, such as a hydantoin, e.g., N,N"-methylene-bis[N'-(hydroxymethyl)-2,5-dioxo-4-imidazolidinyl]urea, N'-(hydroxymethyl)-N-(1,3-dihydroxymethyl-2,5-dioxo-4-imidazolidinyl]-N'-(hydroxymethyl)urea, and Quaternium-15. Preferred compounds are alkanol substituted dialkyl hydantoins having formula V:

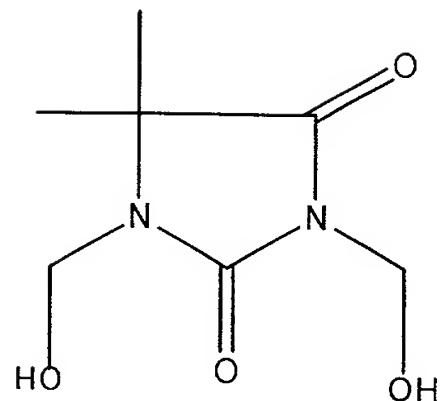


(V)

wherein R<sub>1</sub> and R<sub>2</sub> are each independently hydrogen or (CH<sub>2</sub>)OH, with the proviso that both R<sub>1</sub>

10 and R<sub>2</sub> cannot be hydrogen, and R<sub>3</sub> and R<sub>4</sub> are each independently hydrogen, a methyl group, an ethyl group, a propyl group, or an aryl group.

Alkanol-substituted dimethylhydantoin compounds are preferred formaldehyde donors. A preferred formaldehyde donor of formula (V) is dimethyol dimethyl hydantoin:

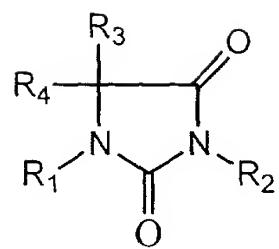


Alkanol-substituted dimethylhydantoin compounds are those described in U.S.

Patent Nos. 3,987,184 and 4,172,140. These are condensation products of 5,5-dimethylhydantoin with one or more moles of formaldehyde (e.g., 1,3-dimethylol-5,5-dimethylhydantoin, 1-methylol-5,5-dimethylhydantoin, or 3-methylol-5,5-dimethylhydantoin and 1-methylol-3-methyloxymethylene-5,5-dimethylhydantoin, and mixtures thereof). Mixtures of alkanol-substituted DMH compounds can also be used. Other formaldehyde donors include n-hydroxymethyl-ureas such as imidazolinyl urea and diazolidinyl urea, diaminomethanes, 1,3-oxazolidines, quaternary hexaminium salts such as Quaternium 15, C-methylols, such as Bronopol, 2-bromo-2-nitro-propan-1-ol, and *O*-hydroxymethyl compounds and formals.

### Stabilizers

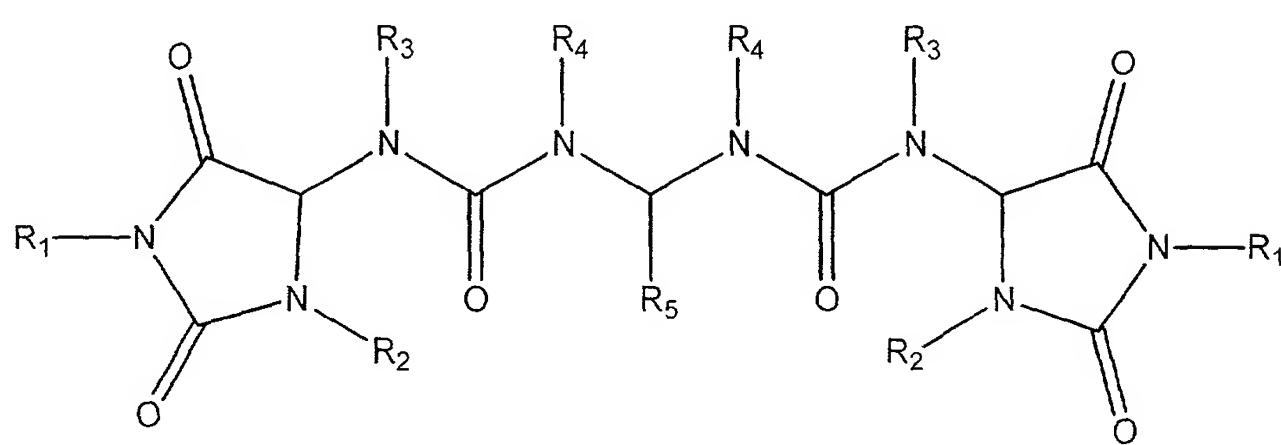
The formulations of the invention also optionally comprise a stabilizer. Suitable stabilizers which may be used in the formulations including hydantoins, ureas and derivatives thereof. The hydantoins are represented by formula V:



(VI)

where R<sub>1</sub> to R<sub>4</sub> are independently selected from H, and a C<sub>1</sub> to C<sub>22</sub> alkyl group.

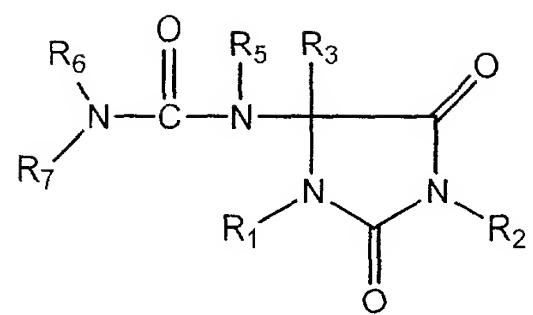
The N,N"-Methylenebis[N'-2,5-dioxo-4-imidazolidinyl]urea and its derivatives are represented by formula VII:



VII

where R<sub>1</sub> to R<sub>5</sub> are independently selected from H or C<sub>1</sub> to C<sub>22</sub>.

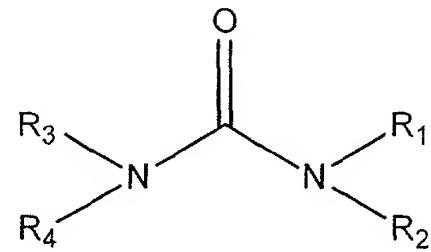
5 The 2,5-Dioxo-4-imidazolidinyl urea (5-ureidohydantoin) and its derivatives are represented by formula VIII:



(VIII)

where R<sub>1</sub> to R<sub>7</sub> are independently selected from H, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub> or C<sub>3</sub>H<sub>7</sub>.

Urea and its derivatives are represented by formula IX:



(IX)

where R<sub>1</sub> to R<sub>4</sub> are independently selected from H or C<sub>1</sub> to C<sub>12</sub>. Where all the R groups are H, the compound is urea.

Preferably, the stabilizer is 5,5-dimethylhydantoin or methylethylhydantoin (MEH).

5                   Stabilizers are preferred in compositions of the invention comprising isothiazolone derivatives.

### Formulations

10                  The preservative concentrates of the invention can be readily prepared in accordance with procedures well known to those skilled in the art, simply by mixing the various components and adjusting the pH using any organic or mineral acid (e.g., hydrochloric acid and acetic acid) suitable for the user's purpose. The manner in which the components are mixed can be modified to suit the needs of the formulator, as discussed below, without departing from the spirit of the invention.

15                  For example, in one method of forming the compositions of the invention, a halopropynyl compound is first dissolved in a butoxydiglycol solvent, and the composition is then mixed until the solution becomes clear. Subsequently, other components of the formulation (for example, an isothiazolone derivative; a hydantoin formaldehyde donor, such as an alkanoldimethylhydantoin; and a stabilizer) can be added. Any water-based additive can be added to the halopropynyl-butoxydiglycol solution.

20                  Because the halopropynyl compound dissolves easily in butoxydiglycol, the preparation of the composition of the invention does not require any heating steps, and the

dissolution can occur at room temperature. Prior art methods involving solvents such as propylene glycol required a heating step to dissolve the halopropynyl compound.

The concentration of the active compounds in the use depends on the nature of the microorganisms to be combated and the composition of the final product to be preserved. For example, the optimum amount of preservative to use for preserving an aqueous composition can be determined by means of screening tests known in the art.

Typically, the components of the composition of the invention are added to personal care, household, and industrial products in amounts such that the final concentration of components remain in the following amounts: (1) for a formaldehyde donor (preferably an alkanoldimethylhydantoin) about 10 to about 800 ppm, preferably between about 10 and about 500 ppm; (2) for a halopropynyl from about 1 to about 100 ppm, preferably from about 2 to about 50 ppm; and (3) for an isothiazolone derivative from about 0.01 to about 5.0 ppm, preferably from about 0.01 to about 2 ppm. The remainder of the composition may be butoxydiglycol, and/or optionally other solvents.

The preservative of the invention is useful for combating microorganisms and, in particular, for the preservation of household, industrial and personal care products, such as cosmetics, lotions, creams, deodorants, shampoos, and soaps. Personal care products include any product that is applied to or contacted with the body of humans or animals in normal use. The following is a list of products that can benefit from incorporation of the preservative system of the invention but is not intended to limit the invention thereto: adhesives, including starch, paper and cardboard, textiles, leather, wood and wood products, paints and articles made of plastic, all purpose liquid cleaners, liquid dishwashing detergent, automotive cleaner, surfactant solutions,

5

household polishes, automotive wax, air freshener, carpet shampoo, pre-spotter, liquid laundry products, pesticide for growing crops, non-food fungicide, non-food herbicide, non-food insecticide, non-food repellent, non-food biopesticide, anti-tarnish products, pre-moistened sponges, pre-moistened mops, clay slurries, coatings, polymer emulsion, natural latex, mineral slurries, pigment slurries, water-based building compounds, caulk, sealer, metal working fluids, metal cleaning fluids, hydraulic fluids, electrodeposition fluids, industrial process water, air washer systems, oil field injection water, liquid hydrocarbon fuels, industrial recirculating cooling water, lubricants, and other materials which can be attacked or decomposed by microorganisms.

10  
15

Microorganisms which effect contamination or degradation of products include bacteria, fungi, yeasts, algae, and slime. Microorganisms of the following genera are examples: *Alternaria*, such as *Alternaria tenuis*, *Aspergillus*, such as *Aspergillus niger*, *Chaetomium*, such as *Chaetomium globosum*, *Candida*, such as *Candida albicans*, *Lentinus*, such as *Lentinus tigrinus*, *Penicillium*, such as *Penicillium glaucum*, *Trichophyton*, such as *Trichophyton mentagrophytes*, *Aureobasidium*, such as *Aureobasidium pullulans*, *Enterobacter*, such as *Enterobacter gergoviae*, *Trichoderma*, such as *Trichoderma viride*, *Escherichia*, such as *Escherichia coli*, *Pseudomonas*, such as *Pseudomonas aeruginosa* and *Burkholderia cepacia*, and *Staphylococcus*, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*.

20

Preservative formulations of the invention can also be used directly as they are manufactured without dilution, or in any other manner traditionally used in manufacturing, such as by metering.

## EXEMPLARY EMBODIMENTS OF THE INVENTION

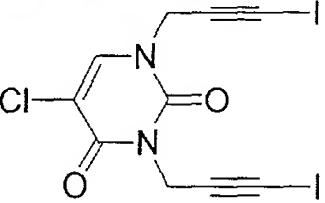
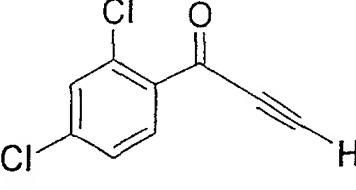
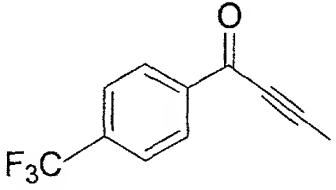
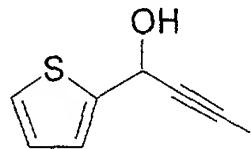
The following Examples are provided to further teach the invention and are not intended to limit the scope thereof. All parts and percentages are given by weight unless otherwise indicated.

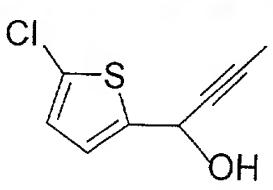
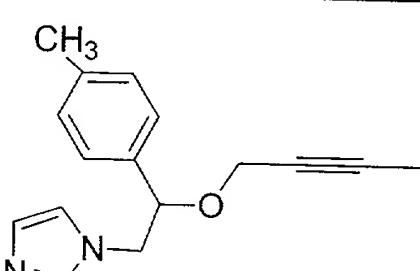
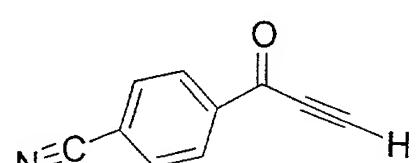
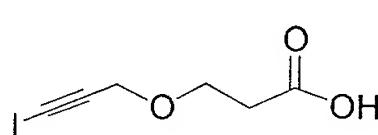
5

### Example 1 - Solubility Data

Several propynyl compounds (including halopropynyl compounds) were tested for solubility in butoxydiglycol. The compounds used in Example 1 are identified below in Table 1.

**Table 1 - Compounds**

Compound	Chemical name (IUPAC)	Chemical structure
L58	1N,3N-Diiodopropyn-2-yl-5-chlorouracil	
L64	1-(2,4-dichlorophenyl)-2-propyn-1-ol	
L74	3-iodo-1-(4-trifluoromethylphenyl)-2-propyn-1-one	
L90	2-(3-iodo-2-propyn-1-ol-yl)thiophene	

Compound	Chemical name (IUPAC)	Chemical structure
L100	5-Chloro-2-(1-hydroxy-3-iodo-2-propyl)thiophene	
L103	2-(1-imidazolyl)-1-(4-methylphenyl) ethyl iodopropargyl ether	
L106	1-(4-cyanophenyl)-2-propyn-1-one	
L111	Iodopropyloxypropionic Acid	

The results of the solubility testing is shown below in Table 2. A “+” indicates that the propynyl compound is soluble, whereas a “-” indicates that the propynyl compound is insoluble. An “x” indicates that the solubility test was not conducted for that combination of propynyl compound and solvent.

L58, L74, L90, L100, L103 and L111 are halopropynyl compounds.

5 **Table 2 - Solubility Data for Propynyl Compounds**

SOLVENT	COMPOUND					
	L103	L90	L106	L58	L74	L64
Butanediol Anhydrous	-	+	+	-	+	+
Sorbitol	-	-	-	x	x	x
Butoxydiglycol	+	+	+	+	+	+

Table 2 demonstrates that the propynyl compounds are soluble in butoxydiglycol.

10 **Example 2 - Testing of Antifungal and Antibacterial Properties  
of Solutions of Propynyl Compounds**

15 Each of the samples A to E is a protein shampoo comprising a propynyl compound. The samples were tested for antibacterial and antifungal properties by testing for inhibition of a mixed bacteria, comprising *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), and *S. aureus* (ATCC 6538), and inhibition of a mixed fungi, comprising *A. niger* (ATCC 16404) and *C. albicans* (ATCC 10231). The results are depicted below, in Table 3.

20 The samples "GPL" and "NP" are controls. "GPL" is 0.1% Glydant® Plus, a liquid antimicrobial preservative available from Lonza Inc., Fair Lawn, New Jersey, containing a dimethyol dimethyl hydantoin formaldehyde donor. Glydant® Plus is used in personal care formulations in cosmetics and personal care products. "NP" is a protein shampoo which does not contain any preservatives.

25 In Table 3, samples A and E consist of an iodopropynyl compound in a butoxydiglycol solvent. Samples C and D consist of an iodopropynyl compound in a butylene glycol solvent, and Sample B consists of a propynyl compound in a butylene glycol solvent.

**Table 3 - Screening of Halopropynyl Solutions for Antifungal and Antibacterial Properties**

Sample	Conc. (ppm)	DAY 0		DAY 14		DAY 28	
		Bacteria (cfu/ml)	Fungi (cfu/ml)	Bacteria (cfu/ml)	Fungi (cfu/ml)	Bacteria (cfu/ml)	Fungi (cfu/ml)
A (L 58)	500	8.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	<10	<10	<10	<10
B (L 64)	500	8.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	>3.0 x 10 <sup>3</sup>	<10	>10 <sup>3</sup>	>10 <sup>3</sup>
C (L 74)	500	8.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	>3.0 x 10 <sup>3</sup>	<10	>10 <sup>3</sup>	>10 <sup>3</sup>
D (L 90)	500	7.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	>3.0 x 10 <sup>3</sup>	<10	>10 <sup>3</sup>	>10 <sup>3</sup>
E (L 103)	500	7.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	<10	500	<10	400
GPL	1000	9.00 x 10 <sup>6</sup>	2.00 x 10 <sup>5</sup>	<10	<10	<10	<10
NP	-	9.00 x 10 <sup>6</sup>	2.00 x 10 <sup>5</sup>	9.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	9.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>

“Cfu/ml” represents colony forming units per milliliter.

The results in Table 3 show that the halopropynyl compounds dissolved in butoxydiglycol have unexpected antibacterial properties.

**Example 3 - Testing of Antifungal and Antibacterial Properties of Halopropynyl Compounds in Protein Shampoo Formulations**

Samples of a standard protein shampoo (pH - 7.0) were formed. The shampoo contained the following preservative-free ingredients, w/w%

Sodium lauryl ether	35.0%
triethanolamine lauryl sulfate	25.0%
cocomide DEA	3.0%
anhydrous protein	1.0%
sterile DI water	36.0%

10% citric acid 0.3%

Samples A, F and H contained active preservative iodopropynyl agents in a solvent. Samples A and H contained butoxydiglycol as the solvent. Samples F and G (containing a propynyl compound rather than a halopropynyl compound) contained butylene glycol as the solvent. Results of the antifungal and antibacterial testing are depicted in Table 4 below.

5 **Table 4 - Antifungal and Antibacterial Screening of Propynyl Compositions in Protein Shampoo Formulations**

Sample	Cone. (ppm)	DAY 0		DAY 14		DAY 28	
		Bacteria (cfu/ml)	Fungi (cfu/ml)	Bacteria (cfu/ml)	Fungi (cfu/ml)	Bacteria (cfu/ml)	Fungi (cfu/ml)
A (L58)	500	9.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	<10	<10	<10	<10
F (L100)	500	8.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	2.00 x 10 <sup>1</sup>	<10	200	<10
G (L106)	500	8.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	>1.0 x 10 <sup>4</sup>	>1.0 x 10 <sup>3</sup>	<10	100
H (L111-01)	500	8.00 x 10 <sup>6</sup>	8.00 x 10 <sup>6</sup>	<10	<10	<10	<10
GPL	1000	9.00 x 10 <sup>6</sup>	2.00 x 10 <sup>5</sup>	<10	<10	<10	<10
NP	-	9.00 x 10 <sup>6</sup>	2.00 x 10 <sup>5</sup>	9.00 x 10 <sup>6</sup>	1.00 x 10 <sup>5</sup>	9.00 x 10 <sup>6</sup>	1.00 x 10 <sup>4</sup>

10 These results demonstrate that halopropynyl compounds in a butoxydiglycol solvent have strong antibacterial and antifungal efficacy. The data indicates that the combination of a halopropynyl and a butoxydiglycol solvent may have commercial utility as an antibacterial and antifungal, broad spectrum preservative.

#### **Example 4 - Testing of Minimum Inhibitory Concentration**

The following example demonstrates the synergistic properties of IPBC and butoxydiglycol solvent against the bacteria *Burkholderia cepacia*. Sample protein shampoos I, J and K were formed. Sample I contained only IPBC. Sample J contained only butoxydiglycol. 5 Sample K contained both IPBC and butoxydiglycol.

The results show that either 1,250 ppm of IPBC or 12,500 ppm of butoxydiglycol is required to completely inhibit growth of *B. cepacia* (samples I,J). However, when IBPC and butoxydiglycol are combined in a single formulation, only 312 ppm of IPBC and 3,250 ppm of butoxydiglycol inhibit bacterial growth.

**Table 5 - Synergy between IPBC and Butoxydiglycol Against *Burkholderia Cepacia***

Sample	ACTIVE INGREDIENT		MIC, ppm active
	IPBC	Butoxydiglycol	
I	+	-	1,250
J	-	+	12,500
K	+	+	312 (IPBC)/ 3,250 (butoxydiglycol)

15 MIC is the “minimum inhibitory concentration,” or the minimum concentration which completely inhibits microbial growth. A “+” indicates that the active is present in the sample, whereas a “-” indicates that the active is not present in the sample.

The data in Table 5 demonstrates that the presence of a halopropynyl such as IPBC and butoxydiglycol solvent has synergistic properties, i.e., the presence of the 20 butoxydiglycol increases the antibacterial efficacy of the halopropynyl compound.

The data in Table 5 also demonstrates the broader efficacy demonstrated by formulations containing a fungicidal halopropynyl compound and butoxydiglycol. The formulations of the invention unexpectedly demonstrate antibacterial properties, in addition to the known fungicidal properties of the halopropynyl compound.

5 Synergism for the IPBC/butoxydiglycol studies in Table 5 was calculated by the method described in C.E. Kull et al., "Mixtures of Quaternary Ammonium Compounds and Long-Chain Fatty Acids as Antifungal Agents", *Applied Microbiology*, 4:538-541 (1961). The Kull method determines the quantity of each component in a mixture which is required to reach a given microbiological endpoint (e.g., MIC), and compares that amount with the quantity of the pure component necessary to reach the same endpoint. According to this method, a synergism index or value  $Q_A/Q_B + Q_a/Q_b$  is determined, wherein:

10  $Q_a$  is the concentration of IPBC, acting alone, which results in MIC;

15  $Q_b$  is the concentration of butoxydiglycol, acting alone, which results in MIC;

20  $Q_A$  is the concentration of IPBC in mixture with butoxydiglycol, which results in MIC; and

25  $Q_B$  is the concentration of butoxydiglycol in mixture with IPBC, which results in MIC.

When the value of  $(Q_A/Q_a + Q_B/Q_b)$  is less than 1.0, the mixture is synergistic.

Values for  $(Q_A/Q_a + Q_B/Q_b)$  of 1.0 and greater than 1.0 represent an additive effect and an antagonist effect, respectively.

20 The results calculated from the experimental results in Table 5 are shown in Table 6 below.

**Table 6 - Synergism Index for IPBC and Butoxydiglycol Combinations**

Parameter	Concentration (ppm)				Synergism Index $Q_a/Q_A + Q_b/Q_B$
	$Q_A$	$Q_B$	$Q_C$	$Q_D$	
MIC	1,250	12,500	312	3,250	0.51

The synergism index value of 0.51 indicates a synergistic relationship between the halopropynyl compound IPBC and butoxydiglycol.

**Example 5 - Testing of IPBC in Antibacterial Properties of Shampoo Formulations**

The test formulations L and M were prepared by mixing 14 % isothiazolone (CMI/MI 2.8 :1) with Glydant® 2000 (a dimethylol dimethyl hydantoin sold by Lonza Inc., Fair Lawn, NJ), with or without IPBC in protein shampoo. IPBC was preliminary dissolved in butylene glycol at 50° C and subjected to intense stirring for 30 minutes, and then incorporated in protein shampoo. Minimum Inhibitory Concentrations against *Burkholderia cepacia* ATCC 25416 are shown in Table 7.

**Table 7 - Evaluation of Preservative Formulations in Protein Shampoo**

Sample	Active ingredients			MIC
	IPBC, ppm	Glydant®, % product	CMI/MI, ppm	
L	-	+	+	0.1% Glydant® 0.55 ppm (CMI/MI)
M	+	+	+	0.05% Glydant® 0.27 ppm (CMI/MI) 156 ppm IPBC

5 Four protein shampoo test formulations were prepared and tested in protein shampoo: one without the addition of preservative and three with varying amounts of IPBC (a halopropynyl), Glydant® 2000 (a DMDMH formaldehyde donor), CMI/MI (isothiazolone derivatives), and butoxydiglycol. The composition of tested formulations is shown in Table 8.

**Table 8 - Final Concentration of Active Ingredients Used in Test Formulations**

Sample	Concentration in protein shampoo			
	IPBC, ppm	Glydant®, % product	CMI/MI, ppm	Butoxydiglycol
N	-	-	-	-
O	50	17.5	0.06	1000
P	100	17.5	0.06	2000
Q	50	35	0.12	1000
R	100	35	0.12	2000

10 15 The samples N, O, P, and Q and R were tested in a preservative Challenge Test against mixed bacteria comprising *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, and *Escherichia coli* ATCC 8739. The challenge test results are shown in Table 9.

5 **TABLE 9 - Challenge Test Results for Preservative Blend in Protein Shampoo**

Sample	Efficacy, cfu/ml	
	Day 0	Day 7
N	6.0 x10 <sup>6</sup>	8.0 x10 <sup>6</sup>
O	6.5 x10 <sup>6</sup>	<10
P	6.0 x10 <sup>6</sup>	<10
Q	7.0 x10 <sup>6</sup>	<10
R	6.5 x10 <sup>6</sup>	<10

10 Cfu/ml – colony forming units in 1 ml.

15 All patents, patent publications, and literature references cited in this specification  
are hereby incorporated by reference in their entirety.

20 Many variations of the present invention will suggest themselves to those skilled in  
the art in light of the above detailed description. Such obvious variations are within the full scope  
of the appended claims.